510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k103037

B. Purpose for Submission:

New device

C. Measurand:

Urinary nitrite and leukocyte esterase

D. Type of Test:

Qualitative colorimetric assays

E. Applicant:

IND Diagnostic Inc.

F. Proprietary and Established Names:

IND Urinary Tract Infection (UTI) Test Strips

G. Regulatory Information:

Classification Name	Product Code	Device Class	Regulation Number	Panel
Nitrite (non-quantitative) test system	NGJ	I, meets the limitations of exemptions in 21 CFR 862.9 (c)(9)	21§862.1510	(75) Chemistry
Leukocyte peroxidase test	LJX	I, meets the limitations of exemptions in 21 CFR 864.9 (c)(9)	21§864.7675	(81) Hematology

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   The IND Urinary Tract Infection (UTI) Test Strips are intended for qualitative detection of nitrite and leukocytes in urine as an aid in the screening of urinary tract infection in persons with signs and symptoms of urinary tract infection. Testing of urine is performed by urinating into a sample cup and briefly dipping the test strip into it. This test is intended for over-the-counter home use only.

3. Special conditions for use statement(s):

   Over-the-counter (OTC) - The labeling states that there is a possibility of false results and users are to contact their physician before making any medical decisions.

4. Special instrument requirements:

   None required.

I. Device Description:

The device is a urine reagent strip containing test pads for nitrite and leukocyte esterase mounted on a thin plastic strip. The test strips are packaged in individual pouches that contain the comparative color chart. The test strip is dipped into a container of freshly collected urine and, if nitrite and/or leukocyte esterase is present, the color changes on the test strip. Strip colors are compared to color chart on the strip pouch. Each strip is a one-time use, disposable device. The IND Urinary Tract Infection (UTI) Test Strips are for over-the-counter use.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ACON UTI Urinary Tract Infection test Strips

2. Predicate K number(s):

   k063295

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (k063295)
Indications for Use /Intended Use	For the qualitative detection of nitrite and leukocytes in urine as an aid in the screening of urinary tract infection.	Same

Similarities		
Item	Device	Predicate (k063295)
Conditions for Use	Over-the-counter	Same
Specimen	Urine	Same
Test principles	Nitrite: Bacteria can change nitrate into nitrite. Nitrite in the urine first reacts with p-arsanilic acid to form a diazonium compound. The diazonium compound reacts with N-(1-Naphthyl)-Ethylenediamine to produce a consistent pink to red color on the test strip. Leukocytes: The test can reveal the presence of granulocyte esterase. The granulocyte esterase will cleave a derived 3-Indoly-phenol ester and release a product. This product will react with benzendiazonium salt to generate a beige-pink to purple color on the test strip.	Same
Test read time	Nitrite = 1 minute, Leukocytes = 2 minutes	Same
Assay cutoff concentrations	0.05 mg/dL for nitrite, 9-15 cells/ mcL for leukocyte test	Same

Differences		
Item	Device	Predicate (k063295)
Testing Method	Dip	Midstream and dip
Closed vial stability	18 months at room temp.	24 months at room temp.
Interference substances	Nitrite: Sodium Bicarbonate ≥ 941 mg/dL, pH < 5. Leukocyte: Phenolphthalein ≥ 740 mg/dL.	Nitrite: Sodium Bicarbonate ≥ 1,000 mg/dL, Riboflavin ≥ 10 mg/dL, Ascorbic Acid ≥ 30 mg/dL, Specific Gravity ≥ 1.030. , pH ≥ 8. Leukocyte: Riboflavin ≥ 10 mg/dL, Albumin ≥ 1,000 mg/dL, Specific Gravity ≥ 1.025, pH ≥ 8.

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP7-A2; Interference Testing in Clinical Chemistry:Approved Guideline 2nd ed.

L. Test Principle:

Nitrite: Several species of gram negative bacteria can change nitrate into nitrite. Nitrite in the urine first reacts with p-arsanilic acid to form a diazonium compound. The diazonium compound reacts with N-(1-Naphthyl)-Ethylenediamine to produce a consistent pink to red color on the test strip.

Leukocytes: The test can reveal the presence of granulocyte esterase. The granulocyte esterase will cleave a derived 3-Indoly-phenol ester and release a product. This product will react with benzendiazonium salt to generate a beige-pink to purple color on the test strip.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

In-house precision studies were conducted by four technicians, twice daily for 10 consecutive days using 5 lots of IND Urinary Tract Infection (UTI) Test Strips (n=400). The materials tested consisted of contrived urine samples containing 0, 0.1 and 5 mg/dL of nitrite and 0, 15, 70, 125, and 500 leukocytes/mcL. The expected concentrations were quantitatively confirmed by hemocytometer counts for the leukocytes or spectrophotometically using the Griess method for nitrite, and qualitatively confirmed using the predicate. The combined lot results are summarized below:

Nitrite

mg/dL (qualitative)	**Expected**	Neg (-)	0.1 mg/dL (+)	5 mg/dL (++)	**% Exact agreement**
Observed	Neg (-)	400	0	0	100%
	0.1 mg/dL (+)	0	400	0	100%
	5 mg/dL (++)	0	0	400	100%
	Total exact match	400	400	400	
	± 1 color block match		400	400	

Leukocyte

cells/mcL (qualitative)	**Expected**	Neg (-)	15 (±)	70 (+)	125 (++)	500 (+++)	**% exact agreement**	**% ± 1 color block agreement**
Observed	Neg (-)	400					100%	
	15 (±)	2	398				99.5%	100%
	70 (+)			397	3		99.25%	100%

	125 (++)				400		100%	100%
	500 (+++)					400	100%	100%
	Total exact match	400	398	397	400	400		
	± 1 color block match		400	400	400	400		

b. Linearity/assay reportable range:

Linearity across the color block concentration range of the test strips were conducted by four technicians, in quintuplicate for 1 day using 2 lots of IND Urinary Tract Infection (UTI) Test Strips (n=40). The materials tested consisted of contrived urine samples containing 0, 0.1 and 5 mg/dL of nitrite and 0, 15, 70, 125, and 500 leukocytes/mcL. The expected concentrations were quantitatively confirmed by hemocytometer counts for the leukocytes or spectrophotometically using the Griess method for nitrite, and qualitatively confirmed using the predicate device. The combined lot results are summarized below:

Leukocyte

cells/mcL (qualitative)	**Expected**	**Neg (-)**	**15 (±)**	**70 (+)**	**125 (++)**	**500 (+++)**
IND UTI	**Neg (-)**	40				
	15 (±)	1	39			
	70 (+)			37	3	
	125 (++)				40	
	500 (+++)					40
Total exact	**match and (%)**	40 (100)	39 (97.5)	37 (92.5)	40 (100)	40 (100)
± 1 color	**block match and (%)**		40 (100)	40 (100)	40 (100)	40 (100)

Nitrite

mg/dL (qualitative)	**Expected**	**Neg (-)**	**0.1 (+)**	**5 (++)**
IND UTI	Neg (-)	40	0	0
	0.1 (+)	0	40	0
	5 (++)	0	0	40
Total exact	**match and (%)**	40 (100)	40 (100)	40 (100)
± 1 color	**block match and (%)**		40 (100)	40 (100)

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Stability testing was performed on 3 lots of test strips using a negative control and a standard whose concentrations of nitrite and leukocyte compare to the first positive color block on the test strip (0.1 mg/dL nitrite and 70 cells/mcL leukocyte).

Real time stability: Closed pouch test strips are stored at 15-30°C (59-86° F)/50-60% relative humidity (RH) for a minimum of 18 months. Testing was performed weekly for 4 weeks, monthly for 2-6 months, then at 8, 10, 12, 15, and 18 months. Studies have been completed up to 12 months. The testing protocol and acceptance criteria were reviewed and found to be adequate. Testing is ongoing. Recommended closed pouch stability is 12 months at 15-30°C (59-86° F).

Open pouch stability studies were conducted at 15-30°C (59-86° F)/45-60% RH. The sponsor combined conditions to stress the device. The sponsor paired low and high temperatures with low and high humidity conditions and also tested low temperature with high humidity and high temperature with low humidity. The testing protocol and acceptance criteria were reviewed and found to be adequate. The test strips were stable for 4 hours for all conditions. Testing at 15-30°C (59-86° F)/45-60% RH also showed that open vial strips, when exposed to strong light, are stable for 2 hours. The labeling states that strips are to be used immediately after opening the pouch and they should not be exposed to strong light.

Additional storage temperatures were also tested: -10°C (14° F), 2-8°C (36-46°F), 37°C (99° F), and 42°C (102°F). Strips were stable for 3 months for all storage conditions except 42°C (102°F) which had a 1 month stability. The testing protocol and acceptance criteria were reviewed and found to be adequate. Recommended storage in the labeling is 15-30°C (59-86° F).

d. Detection limit:

Sensitivity studies were performed for leukocyte and nitrite for the first color block change by comparing the positivity of each color block to hemocytometer cell counts for leukocytes, and spectrophotometrically using the Griess method for nitrite concentrations. Samples were analyzed by four technicians in quintuplicate for 3 days using 3 lots of test strips (n=180). The detection limit for each analyte is defined as the concentration where 95% of the results are positive. Results are summarized below.

Nitrite

Concentration (mg/dL)	Negative	Positive	% Exact Agreement
0.025	180	0	0
0.05	3	177	98.3
0.06	0	180	100
0.08	0	180	100

0.1	0	180	100

The nitrite sensitivity for the first color block change is 0.05 mg/dL.

Leukocyte

Concentration (cells/mcL)	0 (-)	15 (±)	70 (+)	125 (++)	% Exact Agreement
3.75	180	0	0	0	0
7.5	81	99	0	0	55
9	7	173	0	0	96.1
12	0	180	0	0	100
15	0	180	0	0	100

The leukocyte sensitivity for the first color block change is 9 cells/mcL. Labeling identifies sensitivity as 9-15 cells/mcL.

e. Analytical specificity:

i.) Specificity studies were conducted for 36 potential interferents according to CLSI EP-7A2. Stock solutions for nitrite and leukocytes were prepared either by spiking negative urine with commercially available reagents, or diluting natural urine with high leukocyte count. Two urine pools for nitrite were prepared to 0.1 mg/dL and 5.0 mg/dL, and two urine pools of leukocytes were prepared to 70 cells/mcL and 500 cell/mcL of leukocytes. The concentrations of nitrite were confirmed by spectrophotometry and leukocyte concentrations were confirmed with cell counts. Stock solutions of the 36 interferents were prepared using a nitrite and leukocyte negative urine pool at 20x therapeutic or reference range concentrations. Interference samples were further diluted up to 5 times for the study. These samples were then tested with each of the two nitrite and leukocyte concentrations. The results are obtained by the direct comparison of the reagent strip with the color blocks on the bottle label. Results from the interferent samples were compared to the nitrite and leukocyte samples with no interferents added. If interference was observed on any sample, further dilutions were made to determine the interference threshold. Analyses were performed in triplicate. Interference was defined as:

Interference Type	Analyte Level	Observed Effect
False positive	Negative	Test sample reads any degree of positive in contrast to a negative result in corresponding control sample.
False negative	Positive	Test sample reads negative in contrast to any degree of positive result in corresponding control sample.
Positive interference	Positive	Both test and corresponding control samples are read as positive, but the intensity of test sample is higher than that of control sample.
Negative	Positive	Both test and corresponding control samples are read

interference		as positive, but the intensity of test sample is lower than that of the control sample.

The maximum concentrations of the interferences tested are below:

Substance Type	Substance	Concentration
Compound	Sodium Nitrate	0.3 mg/dL
	Phenolphthalein	986 mg/dL
	Citric Acid	6 mg/dL
	Ammonium Chloride	200 mg/dL
	Lithium Acetoacetate	16.2 mg/dL
	Theophylline	10 mg/dL
	Glycine	450 mg/dL
Antibiotic	Amoxicillin	7.5 mg/dL
	Ampicillin	5.3 mg/dL
	Cephalexin	11.7 mg/dL
	Sulfapyridine	30 mg/dL
	Sulfamethoxazole	40 mg/dL
	Trimethoprim	40 mg/dL
	Levofloxacin	1.8 mg/dL
	Ciprofloxacin	1 mg/dL
	Nalidixic acid	6 mg/dL
	Nitrofurantoin	0.4 mg/dL
Common OTC remedies	Acetaminophen	20 mg/dL
	Ibuprofen	50 mg/dL
Commonly found in urine	Albumin	60 g/L
	Bilirubin	20 mg/dL
	Lactose	226 mg/dL
	Creatinine	5 mg/dL
	Glucose	1000 mg/dL
	Urea	120 mg/dL
	Ascorbic Acid	2.2 mg/dL
	Riboflavin	10mg/dL
	Hemoglobin	2 g/L
Other endogenous analytes	Fructose	18 mg/dL
	Potassium Chloride	37 mg/dL
	Sodium Bicarbonate	1260 mg/dL
	Calcium Chloride	12 mg/dL
	Galactose	15.13 mg/dL
	Sodium Chloride	648 mg/dL
	Sodium Phosphate	25.2 mg/dL
	Oxalic Acid	0.73 mg/dL

False negative results were observed for leukocytes with phenolphthalein concentrations at ≥ 740 mg/dL. False negative results were observed for nitrite with sodium bicarbonate concentrations at ≥ 941 mg/dL. Dose

response testing for both compounds was conducted with the testing being performed in quintuplicate. The results are summarized below:

Interfering Substance	Nitrite	Interfering Substance	Leukocyte
Sodium Bicarbonate ≥945 mg/dL	5/6 positive at 0.1 mg/dL	Sodium Bicarbonate	No effect at tested concentrations
Phenolphthalein	No effect at tested concentrations	Phenolphthalein ≥740 mg/dL	4/6 positive at 70 cells/ul

ii.) The effects of pH and specific gravity on the IND Urinary Tract Infection (UTI) Test Strips were evaluated on 120 strips from three lots using nitrite and leukocyte samples prepared following the same protocol as the study above. Urine pH samples were evaluated in 0.5 increments from 4.5-9.0. Adjustments in pH were made using either NaOH or HCl. Target values were determined using a pH meter. Urine specific gravity samples were prepared by spiking with sodium chloride to ≤ 1.005, 1.010, 1.020, 1.030 and > 1.030. Target values were determined using a hydrometer. Two concentrations of leukocytes, (70 cells/mcL, 500 cells/mcL), and two nitrite concentrations (0.1 mg/dL, 5.0 mg/dL) were evaluated. Target concentrations for leukocytes and nitrites were established by microscopic examination with a hemocytometer, and the Griess method, respectively. No interference was observed for leukocytes across the nitrite, pH, or specific gravity ranges tested. No interference from specific gravity was observed for nitrite. There was a negative interference observed at pH 4.5 and 5, however, all strips still indicated a positive result (see table of definitions above). The labeling states that urine pH ≤ 5 may produce a false negative result for nitrite.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Lay user studies were conducted at 5 point-of-care sites by 126 total study participants and 2 healthcare professionals at each site. The lay use study participants had signs and symptoms of urinary tract infection including burning or pain during urination, frequency, fever, back or groin pain, cloudy, dark or bloody urine, or foul smelling urine. Lay users collected the urine in a cup and performed self testing. They also filled out an ease of use questionnaire. The cup sample and questionnaire were blinded and split into 2 additional cups and given to 2 healthcare professionals (HCP). One HCP tested the sample with the new device and the other HCP tested the sample

with the predicate. The three results were recorded on separate forms to avoid bias. In order to obtain more positive nitrite samples, the sponsor conducted additional studies at 2 more POC sites where testing was performed with the IND UTI Test Strip by lay users and the predicate device by a healthcare professional. (n=133)

Leukocytes: Lay Users vs Predicate (N=133)

Predicate (cells/µL)		**Neg (-)**	**15 (±)**	**70 (+)**	**125 (++)**	**500 (+++)**
Lay User	**Neg (-)**	87	1			
	15 (±)	2	11	1		
	70 (+)		4	10		
	125 (++)			1	12	
	500 (+++)					4
Total exact match and (%)		87 (97.8)	11 (68.8)	10 (83.3)	12 (100)	4 (100)
± 1 color block match and (%)			16	12	12	4

Negative agreement was (87/89) 97.8%, positive agreement was (43/44) 97.7%, false positive was (2/87) 2.2%, and false negative (1/44) was 2.3%.

Nitrites: Lay User vs Predicate

Predicate (qualitative)		**Neg (-)**	**Pos (+, ++)**
Lay User	**Neg (-)**	124	
	Pos (+, ++)		9
Total exact match and (%)		124 (100)	9 (100)
± 1 color block match and (%)			9 (100)

Negative agreement was (124/124) 100%, positive agreement was (9/9) 100%, false positive was (0/9) 0%, and false negative was (0/9) 0%.

Due to the lack of positive nitrite samples, the sponsor also performed an additional lay user study with 80 banked samples that were representative of the intended use population. The samples were masked and divided into sets of 4 samples. After reading the package insert, 20 lay users analyzed 80 banked samples. Two lots of IND Urinary Tract Infection (UTI) Test Strips were used in the study. The lay users compared their results to the color chart in the labeling and recorded their responses. A HCP also performed testing on the masked samples with the predicate device. All samples were analyzed in singlicate according to the labeling and the results were compared to the predicate device. The results are summarized below:

Leukocytes: Lay Users vs Predicate

Predicate (cells/µL)		Neg (-)	15 (±)	70 (+)	125 (++)	500 (+++)
Lay User	**Neg (-)**	40				
	15 (±)		0			
	70 (+)			9		
	125 (++)				14	1
	500 (+++)				1	15
Total exact match and (%)		40 (100)	n/a	9 (100)	14 (93.3)	15 (93.8)
± 1 color block match and (%)			n/a	9 (100)	15	16

The negative agreement and positive agreement of results were both 100%. The false negative and false positive results were both 0%.

Nitrites: Lay User vs Predicate

Predicate (qualitative)		Neg (-)	Pos (+, ++)
Lay User	**Neg (-)**	40	2
	Pos (+, ++)		38
Total exact match and (%)		40 (100)	38 (95)
± 1 color block match and (%)			40 (100)

Negative agreement was (40/40) 100%, Positive agreement was (38/40) 95%, False positive was (0/40) 0%, and False negative was (2/40) 5%.

b. Matrix comparison :

Not applicable. The device is for human urine only.

3. Clinical studies :

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

1) A spiking study was performed with 50 lay users to evaluate the ability of users to correctly perform the test and obtain the expected results. Each user was presented with a blinded set of 5 spiked samples that spanned the color block range for each test pad (n=50 per concentration). Expected values were determined by hemocytometer for leukocytes and by the Griess method for nitrite. The results are summarized below:

Leukocytes: IND UTI vs Expected Results

Expected Concentrations (cells/µL)		**Neg (-)**	**15 (±)**	**70 (+)**	**125 (++)**	**500 (+++)**	**% exact agreement**
Lay User	**Neg (-)**	50	5				100
	15 (±)		40				80
	70 (+)		5	44	1		88
	125 (++)			6	49		98
	500 (+++)					50	100
Total exact match		50	40	44	49	50	
± 1 color block match			50	50	50	50	

Agreement with the expected results: Negative agreement (50/50) 100%, Positive agreement (195/200) 97.5%, False positive (0/50) 0%, False negative (5/200) 2.5%.

Nitrites: IND UTI vs Expected Results

Expected (mg/dL)		**Neg (-)**	**0.1 (+)**	**5 (++)**	**% exact agreement**
Lay User	**Neg (-)**	150			100
	0.1 (+)		50		100
	5 (++)			50	100
Total exact match		150	50	50	
± 1 color block match			50	50	

Agreement with the expected results: Negative agreement (150/150) 100%, Positive agreement (100/100) 100%, False positive (0/150) 0%, False negative (0/150) 0%.

2) Dip time study: A dip time study was performed using positive and negative control solutions. Testing was performed with one strip lot by dipping the UTI test strip for < 2 seconds, 2 seconds, 10 seconds, and >10 seconds in each control solution. Each dip time and control solution were tested at n=10 replicates. The results demonstrate that the device performance was not affected by the dip times tested.

3) A usability study was performed with 126 lay users. Survey results included in the 510(k) demonstrated that users included a spectrum across ages and educational levels. 88% (112/126) thought the instructions were not difficult to understand. 8.7% (11/126) did not respond to the survey.

4) Read time study: Read time studies were performed to determine the effects of inaccurate read times. A negative and positive sample for nitrite and leukocytes were used for the study. After wetting the test strip, the results were read at 15-30 seconds, 1 minute, 3 minutes, and 5 minutes. The test strips provided accurate results up to 3 minutes. At 5 minutes a negative result would become positive for nitrite and the color change for leukocytes increased by one color block. The labeling recommends that the read time for nitrite is 1 minute after wetting the strip, and 2 minutes for leukocytes.

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.